MULTI-PASS ENRICHMENT AND DETECTION OF SURFACE ASSOCIATED ANTIGENS

Field of The Invention

The field of the invention is diagnostic tests.

Background of The Invention

Microorganisms are ubiquitous on almost all surfaces in natural and man-made environments, and a considerable number of microorganisms are directly or indirectly linked to diseases in animals and man. Enrichment and detection of bacterial pathogens is particularly important in the food industry where relatively low amounts of bacterial pathogens may cause severe illness or even death, and various methods have been developed to enrich and identify potential microbial contaminations.

Samples suspected to contain a bacterial pathogen are commonly incubated in one or more than one selective growth medium to enrich, isolate and/or characterize a suspected pathogen. Selective growth media are available for a large number of bacterial strains, and culture conditions and procedures are well established for many microorganisms. Moreover, the test procedures are often very simple and generally require a relatively low amount of technical equipment. However, test results may vary dramatically depending on the method of sample preparation, and where the sample preparation requires enrichment steps, enumeration of the pathogen in the sample may become increasingly difficult. Furthermore, in order to generate bacterial colonies or visually identifiable biochemical transformations in the growth medium that assist in identification of the pathogen, incubation times of typically more than 10 hours are required.

Chapman et al. reported an immunomagnetic separation of *Escherichia coli O157* that reduces the time for specific isolation of a particular bacterial pathogen, using antibody coated magnetic beads in suspension of bovine fecal samples [*A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing Escherichia coli O157 from bovine faeces.* Chapman, P.A., Wright, D.J., and Siddons, C.A.; J. Med. Microbiol. 1994, 40(6): 424-427]. Although immunomagnetic separation is significantly faster than enrichment on

or in selective growth media, considerable time and technical resources are still required to allow sufficient growth and positive identification of a possibly present pathogen.

In an improved method allowing detection of *Escherichia coli* O157:H7 in a sample without enrichment by magnetic beads or selective growth conditions, Tortorello and Steward [*Antibody-direct epifluorescent filter technique for rapid direct enumeration of Escherichia coli O157:H7 in beef.* Tortorello, M.L. and Steward, D.S; Appl. Environ. Microbiol. 1994, 60 (10): 3553-3559] reported an antibody-direct epifluorescent filter technique in which ground beef or beef exudate was trypsinated and filtered through a 0.2μm pore-size black polycarbonate filter. The filter is then stained and developed with fluorescein-labeled anti-O157 antibody and examined under a microscope. Although Tortorello's assay is conceptually relatively simple and significantly faster than selective growth methods, the epifluorescence technique typically requires a trained technician, various additional reagents and a high-power light microscope, all of which increases the cost per assay and typically prevents the assay from being a point-of-use assay. Furthermore, sampling and filter handling may present a significant potential for exposure to contaminated material.

To circumvent at least some of the problems associated with selective and microscopic methods, DeMarco et al. developed a rapid detection system for E.coli O157:H7 using a fiberoptic biosensor [*Rapid detection of Escherichia coli O157:H7 in ground beef using a fiberoptic biosensor*; DeMarco, D.R., Saaski, E.W., McCrae, D.A., Lim, D.V.; J. Food. Prod. 1999, 62(7):711-716], wherein the detection is based on fluorescence emitted from antigen specific antibodies. DeMarco's method provides relatively fast results within about 20min of sampling, and does not necessarily require highly trained workers. Furthermore, the use of a biosensor provides a relatively greater measure of safety for the operator by reducing direct contact with the contaminated sample. However, due to the nature of the fiberoptic probe, only very limited areas of the sample can be tested at a time, and even relatively severe, localized contaminations may not be detected altogether. Furthermore, due to the comparably weak intensity of the excitation light, antigens need to be in close proximity to the biosensor in order to develop fluorescence.

Thus, although various systems to rapidly detect surface associated antigen in samples are known in the art, all or almost all of them suffer from one or more than one disadvantage.

Therefore, there is still a need to provide improved methods and apparatus to enrich and detect surface associated antigens in a sample.

Summary of the Invention

The present invention is directed to methods and apparatus of detecting an antigen on a test surface, in which in one step a roller surface is provided that has a binding agent that specifically binds to the antigen. The test surface is then once or repeatedly contacted with the roller surface to allow the binding agent to bind the antigen, and the bound antigen is subsequently detected on the roller surface.

In one aspect of the inventive subject matter the test surface comprises cells, and is preferably a skin, meat for consumption, or a mucous membrane, while in other aspects the test surface comprises inanimate surfaces including a counter top, a door handle, a toilet seat, or a tile. In particularly contemplated aspects, the roller surface has a cylindrical or spherical shape, and it is especially preferred that the roller surface further comprises microspheres. Both the roller surface and the microspheres are preferably made from cellulose, and further contain monoclonal or polyclonal antibodies as a binding agent.

In another aspect of the inventive subject matter, a method of detecting an antigen associated with a test environment employs a detector surface to which microbeads carrying a binding agent are coupled. The test environment is repeatedly contacted with the detector surface such that a complex between the antigen and the binding agent is formed, and the complex is subsequently detected on the detector surface.

In a still further aspect of the inventive subject matter, an apparatus to detect an antigen on a test surface has a housing with a handle and a contactor. The contactor further has a roller surface that is rotatably coupled to the housing, wherein the roller surface is configured to reciprocally contact the test surface, and it is especially preferred that the apparatus includes a light source and a light detector.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention, along with the accompanying drawing.

Brief Description of The Drawings

Fig. 1A is a flow diagram of a method to detect an antigen proximally associated with a test surface according to the inventive subject matter.

Fig. 1B is a flow diagram of a method to detect an antigen associated with a test environment according to the inventive subject matter.

Fig. 2 is a perspective schematic view of an apparatus to detect an antigen proximally associated on a surface according to the inventive subject matter.

Fig. 3A-C is a cross sectional view of various contactors according to the inventive subject matter.

Detailed Description

As used herein, the term "antigen proximally associated with a test surface" refers to any form of interaction that at least temporarily couples the antigen directly or indirectly to the test surface, wherein the antigen is not completely enclosed by the surface. For example, contemplated interactions include hydrophobic, ionic, or electrostatic forces. In contrast, an antigen that is situated within a cell or dissolved in a fluid is not considered proximally associated to the cell surface or fluid container under the scope of this definition.

As also used herein, the term "test surface" generally refers to any surface that is being tested for the presence of an antigen, and is not limited to a particular material, configuration or structure. For example, contemplated test surfaces may comprise inorganic and/or organic materials, cells, or metals, and particularly contemplated surfaces include biological surfaces such as skin, tissue samples, meat for consumption, etc. Particularly contemplated inorganic surfaces include tiles, counter tops, and toilet seats.

As further used herein, the term "roller surface" refers to any surface on an object that can be rolled on the test surface. For example, the outer surface of a round or multi-sided cylinder is considered a roller surface, because a cylinder can be rolled on a test surface. Other examples for a roller surface include a sphere, which can also be rolled over a test surface. In contrast, a piece of paper is not considered a roller surface, because a flat object can not be rolled.

As still further used herein, the term "specifically binds" refers to a selectivity in binding of at least 100 times, but more preferably at least 10000 times compared to non-specific binding. For example, binding of an antigen to a complementary antibody (*e.g.*, hemoglobin to an anti-hemoglobin antibody), or the interaction between a lectin and a glycosylated C_H portion of an antibody is considered specific binding. In contrast, cyanoacrylate mediated binding of one material to a second material (*e.g.* plastic or glass to 'superglue') is not considered specific binding because of a lack of discrimination between binding of cyanoacrylate to the glass or plastic.

In **Figure 1A**, a method 100A of detecting an antigen proximally associated with a test surface has a step 110A in which a roller surface is provided, wherein the roller surface further comprises a binding agent that specifically binds at least part of the antigen. In a subsequent step 120A, at least part of the test surface is contacted with at least part of the roller surface such that the binding agent binds the antigen to form a bound antigen, and in another subsequent step 130A the bound antigen is detected on the roller surface.

In a preferred embodiment, the test surface is the surface of a beef tenderloin in a meat processing plant. The roller surface is the outer surface of a plastic cylinder with a diameter of 2cm and a length of 6cm, which is coated with microcrystalline cellulose. Covalently bound to the cellulose surface are monoclonal antibodies against a first surface antigen of the bacterium *E.coli* O157:H7 in a total amount of 1mg via a bifunctional linker (e.g., α,ω-bifunctional linker). The antibody-coated cylinder is rolled about five to ten times along the longitudinal axis of the tenderloin using moderate pressure to maintain physical contact between the cylinder and the meat, wherein the speed of rolling is between about two to ten cm/s to allow formation of the antibody-antigen complex. The antibody-*E.coli* O157:H7 complex is incubated *in situ* on the cylinder by rolling the cylinder in a solution of a second fluorescent antibody (0.1mg/ml, FITC labeled, approximately 10ml) with binding specificity to a second *E.coli* O157:H7 surface antigen. After binding of the second fluorescent antibody to the second surface antigen and washing away unbound excess second fluorescent antibody using PBS (50mM phosphate buffered saline, pH7.5, approximately 20ml) the cylinder is irradiated with excitation light of 488nm with an argon laser, and emission light of 530nm is detected using a bandpass filter and a photomultiplier.

In alternative aspects of the inventive subject matter, it is contemplated that the test surface need not be limited to beef tenderloin. In fact, appropriate test surfaces include all surfaces where the detection of a particular antigen is a concern, so long as the test surface may be contacted at least once with the roller surface. Therefore, contemplated surfaces include both non-synthetic and synthetic surfaces. For example, where a bacterial or viral infection of a patient is a concern, the non-synthetic surface may be skin, a finger or toenail, or a mucous membrane. Alternatively, where control of bacterial spoilage of food is a concern, the non-synthetic surface may be the surface of raw or processed meat, fish, fruit, dairy products, or vegetables. Contemplated synthetic surfaces include surfaces comprising metals, polymers, or glass, and especially contemplated synthetic surfaces are counter tops, door handles, tiles, and toilet seats.

It should also be appreciated that the roller surface need not be restricted to a microcrystalline cellulose coated outer surface of a plastic cylinder with a diameter of 2cm and a length of 6cm, and contemplated alternative roller surfaces may vary considerably in size and shape. For example, where accessible test surfaces are relatively small, cylinder- or sphere-shaped roller surfaces with areas of about $3mm^2$ to $5cm^2$ may be employed, whereas for relatively large accessible test areas roller surfaces of about $5cm^2$ to $50cm^2$ and larger may be employed. With respect to the shape or configuration of the roller surface it should be appreciated that appropriate roller surfaces need not be restricted to a sphere or cylinder, but may vary substantially so long as alternative roller surfaces are closed in themselves in at least one dimension. For example, to better accommodate the shape of a particular test surface, appropriate roller surfaces may be complementary in their shape.

Although it is generally preferred that a test surface is contacted with only a single roller surface, it should be appreciated that contacting the test surface with multiple roller surfaces is also appropriate. Multiple test surfaces may be especially advantageous, where more than one antigen is to be detected. Alternatively, where quantification of an antigen is desirable, multiple roller surfaces may be utilized to provide calibration.

With respect to the material, it should be appreciated that the inventive subject matter need not be limited to microcellulose-coated plastic, but various alternative materials are also appropriate. Contemplated alternative materials include natural and synthetic polymers, metals, and any reasonable combination thereof, so long as a binding agent can directly or indirectly be

affixed thereto. For example, where lightweight and durability are especially desirable, aluminum or titanium is contemplated. Where disposable materials are desirable, cardboard or soft plastic may be employed.

Furthermore, it is contemplated that the total surface area of the roller surface may be increased by immobilization of microspheres on the roller surface, and it is especially preferred that such microspheres are cellulose microspheres. Contemplated microspheres may thereby have a size of between about 1μm and 1mm and more, and it should be appreciated that appropriate microspheres may or may not be pre-coated with a binding agent.

In further alternative aspects of the inventive subject matter, the binding agent may be various agents other than a monoclonal antibody. For example, in cases where a monoclonal antibody is not available or where reduction of cost is an objective, a polyclonal antibody preparation may be employed. It should also be noted that the term "antigen" as used herein not only refers to a molecular entity that can be specifically bound by an antibody, but also to molecular entities that can be specifically bound by non-antibody molecules and some of the contemplated sources of antigens may therefore include nucleic acids, organic and organometallic polymeric and non-polymeric compounds, and various epitopes on viruses, bacteria, yeast, mold, spores, and cells. For example, viral antigens may belong to a herpes virus, a hepatitis virus, or a HTLV virus, bacterial antigens may to Staphylococcus aureus, Staphylococcus epidermidis, Helicobacter spec., etc, and contemplated yeast antigens include candida spec. Therefore, depending on the particular nature of the antigen, contemplated binding agents may include lectins, peptides, nucleic acids, chelators, etc. Consequently, the attachment of the binding agent to the roller surface may be considerably different from the bifunctional linker. For example, where no CO_2H or NH_2 functionalities are available, alternative covalent bonds may be formed between reactive groups on the antigen and the binding agent, and contemplated bonds include disulfide bonds, carbon-carbon bonds, etc. Furthermore, it is contemplated that the attachment between the roller surface and the binding agent may be reversible, which may be especially advantageous in cases where the roller surface is to be reused.

In still further alternative aspects of the inventive subject matter, the antibody-coated cylinder need not necessarily be rolled between 5 and ten times over the test surface, but may include more or less frequent contact. For example, where a relatively high density of antigen is

expected, a single pass of the cylinder may be sufficient, while in other cases where a relatively low density of antigen is suspected11 to 50 and more passes may be necessary to form a satisfactory amount of antigen-antibody complex. It should be especially appreciated that by repeatedly applying a roller surface to a test surface, the antigen accumulated on the roller surface, and only a relatively small roller surface compared to a relatively large test area is required to produce a detectable signal. The accumulation of the antigen on the roller surface may thereby be performed by repeatedly passing the roller surface over the same test surface or over different portions of the test surface. Therefore, mainly depending on the amount of binding agent on the roller surface and on the particular binding kinetics of the binding agent, the ratio of delimiting surface to test surface may lie between 1:1 and 1:1000. With respect to the rolling speed it is contemplated that various rolling speeds other than between 2-10cm/s are appropriate. For example, where the radius of the cylinder is relatively small, lower speeds between 0.1cm/s and 2cm/s are contemplated, while cylinders with larger radius may be moved at a speed of 10-30cm/s and faster.

With respect to the detection of the bound antigen on the roller surface it is contemplated that various methods other than employing a FITC-labeled second antibody specific to a second antigen are also appropriate, and contemplated alternative methods include chromogenic reactions which may or may not be mediated by an enzyme linked to the second antibody, radio-labeled second antibody, PCR-based methods, etc. Where the detection involves binding of a second antibody to a second antigen, it is preferred that the step of detection further involves a washing step to remove unbound second antibody. Therefore, contemplated methods of detection may include radiometric detection, fluorescence detection, spectrophotometric detection, etc. It should further be appreciated that the detection may not only be qualitatively, but also be quantitatively.

In **Figure 1B**, a method 100B of detecting an antigen associated with a test environment has a step 110B in which a detector surface is provided, wherein a plurality of microbeads are coupled to the detector surface, and the plurality microbeads further comprises a binding agent that specifically binds at least part of the antigen. In a subsequent step 120B, the test environment is repeatedly contacted with the detector surface such that a complex is formed between the binding agent and the antigen. In a further step 130B the bound antigen is detected on the roller surface.

In a preferred embodiment, the test environment is the skin of a human suspected of carrying the skin pathogen *Propionibacterium acnes* on his or her facial skin. The detection surface is a flat, 5cm x 5cm cellulose swipe, which is coated with cellulose microbeads having an average diameter of 1µm. Covalently bound to the microbeads are rabbit polyclonal antibodies against *Propionibacterium acnes* antigens in a total amount of 1mg via a bifunctional linker (e.g., α,ω-bifunctional linker). The detection surface is swiped about five to ten times across a selected area (*e.g.*, cheek) using moderate pressure to maintain physical contact between the detection surface and the facial skin. The antibody-*Propionibacterium acnes* antigen complex is subsequently incubated *in situ* on the detection surface by immersing the detection surface in a solution of polyclonal anti-*Propionibacterium acnes* fluorescent antibody (0.1mg/ml, FITC labeled, approximately 10ml). After binding of the fluorescent antibody to the bound *Propionibacterium acnes* and washing away unbound excess fluorescent antibody using PBS (50mM phosphate buffered saline, pH7.5, approximately 20ml) the detection surface is irradiated with excitation light of 488nm with an argon laser, and fluorescence emission light is detected at of 530nm using a bandpass filter and a photomultiplier.

It should be especially appreciated that the use of antibody-coated microbeads coupled to the surface of a detection surface substantially increases the total surface, thereby increasing the antibody-load and binding capacity amount of the detection surface. Furthermore, where the microbeads have an average diameter between about 100µm to 1000µm, the detection surface exhibits an abrasive character, which may be particularly advantageous where target antigens are at least partially obstructed by relatively loose objects/particles (*e.g.*, stratum corneum or dead cells). In cases where the microbeads have an average diameter between about 1µm to 1000µm, it should also be appreciated that the detection surface develops an increased capillary effect, which may especially in combination with additional fluids allow a facilitated formation of an antigen-binding agent complex by providing a stationary liquid environment.

In alternative aspects of the inventive subject matter, it is contemplated that the test environment need not be restricted to the facial skin of a human, and various alternative test environments include fluids, synthetic and non-synthetic surfaces, animals and plants, so long as alternative test environments can repeatedly be contacted with the detection surface. For example, fluids such as a beverage or a water sample can repeatedly be contacted by successive immersion of the same detection surface into the fluid. In another example, contemplated synthetic and non-

synthetic surfaces are walls, handles, and soil, while animals and plants include pets, livestock, vegetables, and fruit.

It should further be appreciated that the detection surface need not be limited to a flat, 5cm x 5cm cellulose swipe, and various alternative surfaces are contemplated so long as a plurality of microbeads may be coupled to the surface. Therefore, contemplated surfaces include natural and synthetic polymers, and metals. For example, where the microbeads are latex microbeads, the surface may also be manufactured from latex. In cases where the surface needs to be mechanically resistant, aluminum or stainless steel may be employed. Likewise, the microbeads need not be limited to cellulose microbeads, and contemplated microbeads may be manufactured from organic and inorganic polymers such as latex or glass. With respect to the number or density of the microbeads on the detection surface, it is contemplated that a wide variety of numbers and sizes are appropriate. It is contemplated that generally 5% - 85% of the detection surface is covered with the microbeads, however densities of 0.1% - 4.9% and less are also appropriate. Conversely, where a very high density of microbeads is required densities of 86% - 100% are contemplated. Although preferred microbeads have a size between about 1μM and 1000μm, sizes of between 10nm and 990nm and less are also contemplated, and in cases where the detection surface is relatively large microbeads may have an average diameter of about 1mm to 5mm, and more.

With respect to the antigen it is contemplated that in alternative aspects of the inventive subject matter the antigen need not be restricted to a *Propionibacterium acnes* antigen, but contemplated antigens may include all molecular, macromolecular and larger structures that specifically can be bound by a binding agent with a substantial affinity. The term "substantial affinity" as used herein refers to a binding interaction having a dissociation constant K_D of less than 10^{-4}M. Thus, contemplated antigens include domains of a molecule, entire molecules, polymers, viruses, bacteria, and cells.

It should further be appreciated that there are many modes of contacting the test environment with the detection surface other than swiping without departing from the inventive concept presented herein, and appropriate modes include padding, rubbing, circling, dipping, etc., whereby the test environment may be contacted at the same, or on different locations. For example, where an abundance of antigen is relatively loosely associated with the test environment, a

simple padding contact may be sufficient to allow the formation of an antigen-binding agent complex. In other cases, especially where the antigen is obstructed in the test environment, vigorous rubbing and/or circling of the detection surface may be required. Furthermore, it should be appreciated that the addition of a solvent, or buffer may be employed to dissolve or otherwise separate the antigen from the test environment. Contemplated solvents include benzene, hexane, ethanol, water, or DMSO (dimethylsulfoxide), etc., and buffers may include acidic and basic buffers of various strength and composition (e.g., 1M NaH_2PO_4/Na_2HPO_4 pH8.5, 10mM citrate/citric acid pH3.75).

With respect to corresponding elements between method 100A and method 100B, the same considerations apply for elements in method 100B as discussed in method 100A (*vide supra*).

In **Figure 2**, an apparatus 200 positioned on a test surface 260 has a housing 210 with a handle 212. Partially enclosed in the housing is a contactor 220, which is rotatably coupled to the housing. Contactor 220 has a roller surface 222, onto which a binding agent 224 (not shown in detail) is affixed. The housing further encloses power source 230, signal processor 240, light source 250, and detector 252.

In a preferred embodiment, the apparatus has an overall height of approximately 15cm, a width of about 8cm at its widest point, and a thickness of approximately 4cm at its thickest point. The housing is fabricated from black injection-molded polyvinyl chloride, and the handle (length about 5cm, diameter about 3cm) is an integral part of the housing. The contactor is a cylinder having a roller surface coinciding with the outer cylinder surface, and the contactor is rotatably coupled to the housing via ball bearings in the housing such that the roller surface extends approximately 1/10 radius of the cylinder outwards of the housing to enable reciprocal contact with the test surface. The contactor 220 is made from polyvinyl chloride, has a diameter of about 2cm and a length of approximately 7.5cm, and is coated with microcrystalline cellulose. Attached to the microcrystalline surface is a total of 1mg anti-*E.coli* O157:H7 antibody via a bifunctional linker. Enclosed within the housing is a detection circuitry that includes a rechargeable NiCd battery 230, which is electrically coupled to the signal processor 240, Argon laser 250, and photomultiplier/filter combination 252. All components of the detection circuitry are well known in the art. Test surface 260 is the surface of a beef tenderloin.

Although the housing is preferably sized for manual operation, the housing need not necessarily be limited to any particular size or configuration, so long as the contactor can be rotatably coupled to the housing. For example, in cases where the contactor has a spherical shape, the housing may have a pen-like configuration, whereas in cases where the apparatus has multiple contactors the housing may have a box-like configuration. With respect to the material, it is contemplated that various materials other than black injection-molded polyvinyl chloride are also appropriate, including natural and synthetic polymers, metals, and any reasonable combination thereof. For example, where autoclaving or radiation sterilization is desirable, aluminum or stainless steel may be employed, whereas a disposable apparatus may preferably be manufactured from polyethylene or polycarbonate. Likewise, contemplated apparatus may or may not include an integrated or separate handle.

With respect to the contactor it is contemplated that appropriate contactors may have various shapes other than a cylinder having a diameter of about 2cm and a length of approximately 7.5cm. Contemplated alternative contactors may include various spherical and cylinder-like shapes, so long as they comprise a roller surface. For example, uneven and/or recessed surfaces may advantageously be contacted with a spherical contactor, while rounded surfaces may be contacted with a contactor having a shape complementary to the rounded surface. **Figures 3A-C** shows various examples of vertical cross sections of contemplated contactors 301, 302, and 303 and their corresponding test surfaces 311, 312, and 313, respectively. It should further be appreciated that still other contemplated contactors may be operationally deformable to allow intimate contact between the contactor/roller surface and the test surface. It should further be appreciated that alternative apparatus may have more than one contactor. For example, where multiple antigens are to be detected, 2-5 or more contactors may be utilized.

Although it is preferred that a detection circuitry is included in the housing of contemplated apparatus, it should be appreciated that the detection circuitry may be omitted entirely. For example, where the detection of the bound antigen involves a secondary antibody coupled with an amplifying enzymatic reactions (*e.g.* alkaline phosphatase and p-nitrophenylphenol) the presence of an antigen may be visually confirmed. In alternative aspects, for example for quantitative or semi-quantitative detection, detection need not be restricted to detection of fluorescence, but may include detection of chromophores, radioisotopes, etc. Therefore, the detection circuitry may also comprise a photodiode with or without a filter, a Geiger-Mueller

counter, etc. With respect to the roller surface, the test surface, the binding agent, and the antigen the same considerations as described above apply.

In operation, the detection apparatus is repeatedly passed over the test surface such that the antigen from the test surface is bound by the binding agent on the roller surface. The roller surface with the bound antigen is then incubated (*e.g.*, by rolling) in a solution containing a labeled second binding agent (*e.g.*, a solution with a labeled antibody) that binds to the bound antigen, and the labeled second binding agent is subsequently identified by employing a physico-chemical detection method. It should especially be appreciated that the apparatus according to the inventive subject matter may not only be used to detect the presence of one or more than one antigens on a surface, but also to quantify the antigen. Moreover, the use of a roller surface passing repeatedly over the test surface advantageously increases the area that can be tested by enrichment of the antigen on the roller surface.

Thus, specific embodiments and applications of improved methods and apparatus to enrich and detect surface associated antigens have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms “comprises” and “comprising” should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.